OFFICE OF NAVAL RESEARCH

Annual Progress Report

Report Prepared By: L. L. Waters, M.D.

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ANNUAL RATE: $18,500

CONTRACTOR: Yale University

PRINCIPAL INVESTIGATOR: L. L. Waters, M.D.

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TITLE OF PROJECT: The Pathogenesis of Necrotizing Arteritis

Objectives: The objective of this project has been a clarification of the etiology and pathogenesis of the lesions of chronic vascular disease. The approach to this subject has been through a study of experimental vascular lesions in animals.

SUMMARY OF RESULTS

a. Since start of project:

The investigations carried out under this contract have evolved the basic concept that reactions of the artery wall in any disease, including even arteriosclerosis and rheumatic fever, are qualitatively alike. Such modifications as occur are quantitative, are dependent upon the intensity and duration of the injurious stimulus, and are secondarily imposed upon a local inflammatory response. The basic "inflammatory" reaction of arteries may vary quantitatively in form from inflammation in its broadest

sense - increased local permeability - to the more conventional tissue manifestations of cellular exudate and repair.

At the beginning of the project it was reasoned that if a standard acute arterial lesion could be produced in an experimental animal with regularity, opportunity would be afforded to test the validity of the concept stated above and at the same time to examine etiologic and pathogenetic factors in the establishment of the lesions. Selective necrotizing arteritis of the coronary arteries was produced in dogs by the intravenous injection of allylamine. These lesions have served as test objects for further experimentation because they occur in hours to days, develop in 90 per cent of the test animals, and morphologically reproduce basic acute arterial changes seen in such diseases in man as rheumatic fever, malignant hypertension, and periarteritis nodosa. Similar lesions of the coronary, gastrointestinal, and renal cortical arteries of dogs have been produced for study and for further experimentation by pertinent changes in hemodynamic factors. Thus short episodes of severe hypertension following epinephrine or renin injection lead in dogs to necrosis of coronary and gastrointestinal arteries. Acute alterations in blood volume, effected by transfusions, are followed by the same patterns of arterial lesions. Combination of increased blood volume and hypertensive episodes increase the extent of visceral arterial damage to include renal cortical arteriolar necrosis. These experiments have provided direct experimental evidence of the role of high blood pressure and of altered blood volume in the pathogenesis of arterial damage and describe relatively simple techniques for the further

investigation of such factors in the mechanisms of vascular disease.

With experimental methods available for the rapid production of acute inflammatory changes of the coronary arteries of dogs, the major effort of the project has been to attempt the modification of these lesions towards those of arteriosclerosis in man in order to study the etiology and pathogenesis of this disease. The hypothesis followed in the experiments has been that the characteristic lesions of arteriosclerosis is a basic non-specific inflammatory response of arteries modified by deposit of lipid-rich particles from the ambient blood. This idea has been explored in several series of experiments. It was first shown that India Ink particles injected intravenously localize and are phagocytized selectively at areas of injury (allylamine) in the coronary arteries of dogs. Particles of methyl cellulose, soluble in plasma and of molecular weights ranging from 75,000 to 400,000, localize similarly and result in intimal foam-cellular transformations of the basic acute inflammatory process.

Following these experiments, modification of the basic arterial response was attempted through alteration of the lipid content of the blood of animals with experimentally injured coronary arteries. It was found that fatty, foam-cellular transformation of the basic experimental arterial lesions could be achieved by elevating serum lipids through the intravenous use of detergents (Triton A-20). A more satisfactory and much simpler method for producing fatty, foam-cellular lesions of the coronary arteries has been the intravenous injection of egg-yolk emulsions into allylamine-treated dogs. The arterial changes resulting from this procedure

reproduce many of those found in early arteriosclerosis in man and have allowed study of the deposit of lipid in the vessel wall and its subsequent transport and fate. It should be emphasized that foam-cellular lesions of the aorta and coronary arteries are regularly obtainable in dogs within 10 days of the beginning of the experiment, so that many pertinent variations of either local or systemic factors can be carried out. The extension of this work to a study of the effects of human blood has occupied the project during the present report period and is considered immediately below.

b. During Current Report Period (January 1, 1953 - December 31, 1953):

At the commencement of the past year, the project possessed the information that particulate egg-yolk fat or egg-yolk lipoprotein fractions injected intravenously into allylamine-treated dogs rapidly transformed the basic inflammatory lesion in the coronary arteries into a fatty, foam-cellular granuloma of the arterial intima. At the same time all plasma lipid fractions of the recipient animals were markedly elevated.

It was thought important to extend these observations to a study of the effects of intravenously injected human plasma on the basic allylamine-produced lesions of the coronary arteries. Human plasmas from outdated bloods in the hospital blood-banks were collected in two categories: lactescent (chylomicron rich), and clear (chylomicron poor). The lipids of these plasmas were determined, and they were infused intravenously

into a series of dogs that had been given allylamine to produce standard injury of their coronary arteries. It was found that dogs will tolerate daily infusions of large quantities of citrated human plasma. Amounts given ranged from 30 - 100 cc/K per day. It was noticed that the dogs' own plasma, which normally is practically colorless, became xanthrochromic like human plasma and remained so during the course of injections. Blood lipid fractions rose and cholesterol was maintained in the 200-350 mgm. per cent range. The animals were sacrificed serially after a course of from one to ten injections and their cardiovascular systems examined microscopically. Large quantities of stainable lipid was found deposited in the arterial walls selectively at the sites of allylamine injury. In the dogs given lactascent plasma the amount was greater than in those animals receiving relatively clear plasma, and in the most favorable instances foam-cellular arterial lesions developed. In animals receiving clear plasmas, although less lipid was deposited in the artery walls, the amount was large as compared to allylamine controls.

These experiments have demonstrated that lipid from human plasma is deposited at sites of arterial injury at levels of blood lipid concentration in the upper range of normal for humans. They have raised the question of the relative importance of chylomicrons and of soluble lipoprotein complexes in the genesis of the observed foam-cellular lesions. A series of experiments have been carried out in an effort to clarify this point. The question is important because chylomicrons make up much of the

blood fat in alimentary lipemia whereas soluble lipoproteins maintain basic levels of plasma lipids. A simple method for the separation of a mixed globulin fraction of human blood plasma containing 80-93 per cent of the contained lipid as lipoprotein, has been devised. This method allows preparation of concentrated solutions of the separated lipoproteins. These solutions are suitable for injection in small volume and can be treated conveniently in the high speed centrifuge for the removal of chylomicrons. A detailed description of the method must await necessary study of the conditions of precipitation, of the lipoprotein complexes involved, and of characteristics of the resultant protein solutions. Nevertheless it has been possible to date to inject a small series of allylamine-treated dogs (6) with concentrated lipo-protein solutions, with little contamination by chylomicrons. The plasmas of these animals have been maintained practically particle-free with normal or increased lipid levels. Large deposits of stainable lipid have regularly occurred selectively at the sites of coronary artery injury and foam-cellular transformation of the basic response takes place. The lesions reproduce many of the morphologic features of early arteriosclerosis in man and are well developed within ten days.

These experiments would suggest that soluble lipoproteins of human plasma in concentrations obtaining in plasma in health are associated with the deposit of lipid at sites of arterial injury. They open an experimental approach for the further study of the relation of local and systemic factors

in the establishment of the lesions of chronic vascular disease. Approachable through the same means is a study of factors which might prevent the development of these changes.

PLANS FOR FUTURE:

Immediate:

During the last six months of this project, intensive study of the respective effects of chylomicrons (alimentary lipemia) and of soluble lipoproteins of human blood on the basic inflammatory response of the coronary artery will be continued by the methods described.

Long Range:

None under the auspices of the Navy. This contract is being terminated by Office of Naval Research as of June 30, 1954.

REPORTS AND PUBLICATIONS

(During current report period)

1. L. L. Waters, "Localization of Lipids in Injured Coronary Arteries of Dogs following Injection of Egg-Yolk Fractions or of Hyperlipemic Human Plasma." Circulation 1953, 8, 437.

2. T. O. Gentsch, L. L. Waters, W. W. L. Glenn, "The Influence of Acute Hypervolemia on the Freeze-Dry Homologous Aortic Graft." Surgery 1954, 35, 30.

3. L. L. Waters, "Reaction of the Artery Wall to Injury by Chemicals or Infection." Paper invited by National Research Council for its Symposium on Atherosclerosis, March 1954.

4. L. L. Waters, "Reaction of the Artery Wall to Injury by Hypertension and Hypervolemia." Paper invited by National Research Council for its Symposium on Atherosclerosis, March 1954.